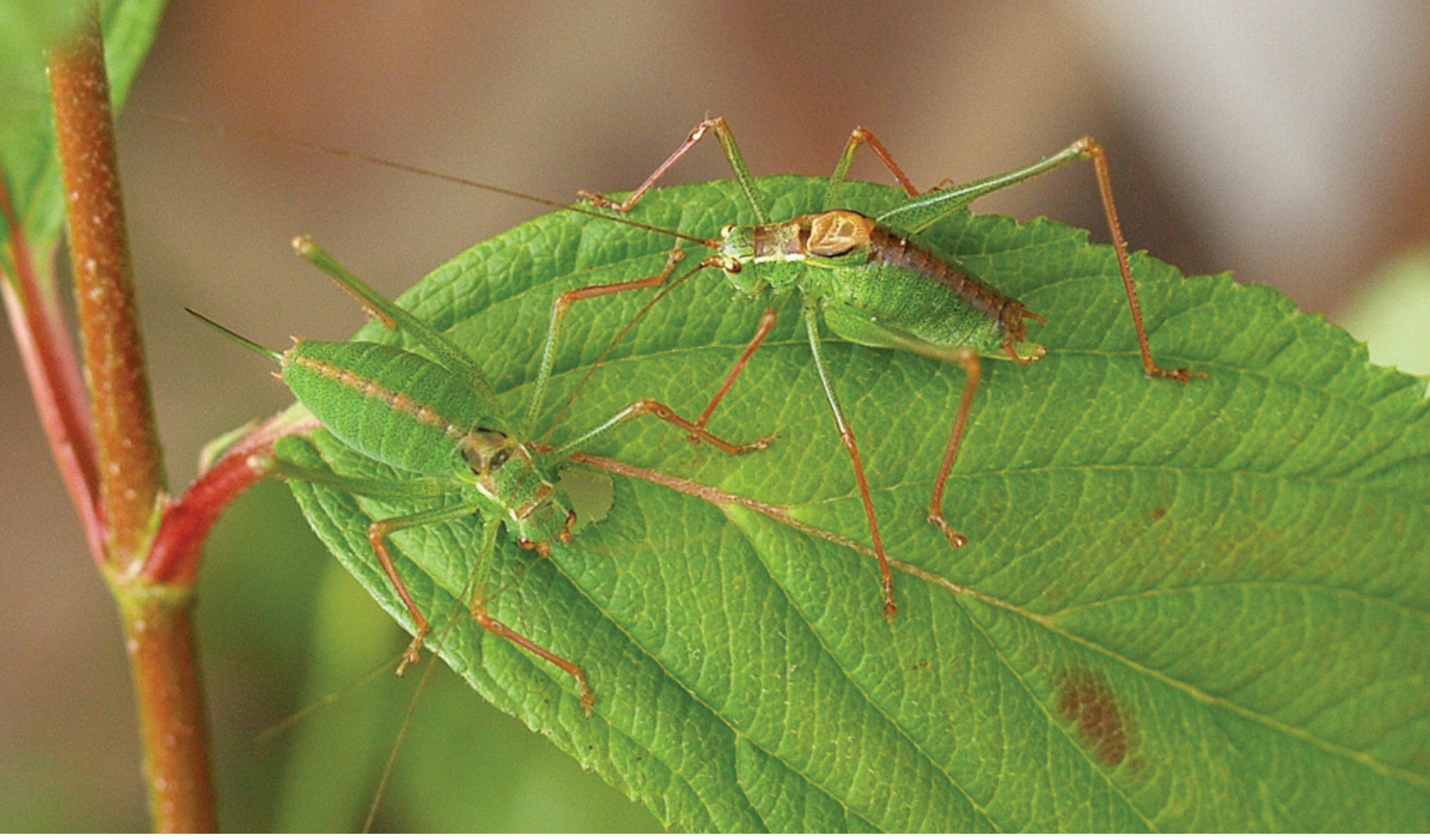
Mating in the speckled bush-cricket, *Leptophyes punctatissima*



Mating in the speckled bush-cricket, *Leptophyes punctatissima*

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Leptophyes punctatissima is a flightless, medium-sized bush-cricket from the Orthopteran family Tettigoniidae, sub-family Phaneropterinae. It was first studied in detail by Duncan (1960) who observed its life cycle in the field and its reproductive behaviour in the lab and gave a brief description of copulation.

We have been working on various aspects of *L. punctatissima* behaviour and acoustics for several decades and have observed its behaviour in over 500 matings both in the lab and in the field. Since we have found very few full accounts of copulation in bush-crickets in the literature (though see Rentz (1972) for shield-backed katydids from the genus *Idiostatus* and Samietz *et al.* (2014) for *Phaneroptera falcata*), one of the main aims of this article is to provide a comprehensive description of copulation behaviour in *L. punctatissima*, based on a synthesis of our various observations.

Leptophyes punctatissima is common throughout Europe. In the UK its distribution is mainly in southern England, though over the last 30 years it has considerably extended its range northwards, and with more populations now established across the Scottish border (NBN Atlas, 2020). It is usually found patchily distributed in mixed vegetation close to trees or tall shrubs. The nymphs are usually found in low vegetation but the adults may move high up into the trees.

Overwintered eggs hatch from early May and the nymphs go through six instars before reaching sexual maturity around early August. Adult females are larger than males (males: mean body length 13.7mm, SD±0.9mm, *N*=79; females: mean body length 15.8mm, SD±1.2mm, *N*=85; recorded from a population reared in the laboratory from about instar 4). Mating tails off after the end of August and most adults are dead by the end of October.

Mating system and calling behaviour

Some bush-crickets have a duetting mating system, especially common among the phaneropterines, in which the male calls and the female responds, though which sex then approaches the other varies (Robinson & Hall, 2002). *Leptophyes punctatissima* is, however, the only UK species where both male and female call in this way. Males call both during the day and at night, with three peaks of calling in the field occurring from midnight to 04.00, from 09.00 to 11.00 and from 14.00-19.00.

Both male and female calls are ultrasonic (~40 kHz) and extremely brief (Robinson *et al.*, 1986; Figure 1). If the female's response falls within a particular time window (Figure 2), the male approaches the stationary female, finding her by orienting to her call (phonotaxis), with the pair continuing to duet throughout his approach (Zimmermann *et al.*, 1989).

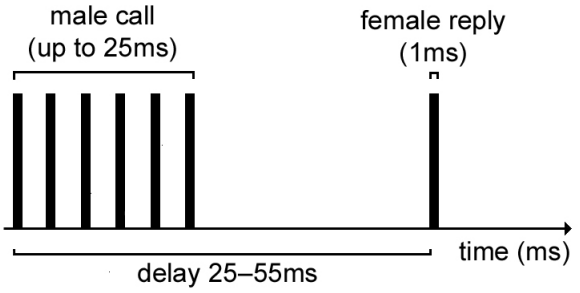

Figure 1. Diagrammatic representation of the timing of the duet. The male call is a brief click consisting of 5-8 syllables and lasting up to 25ms in total. The female call is even briefer with 1-2 syllables, lasting 1-2ms.

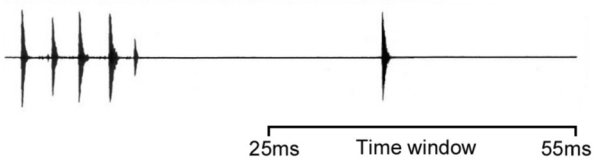

Figure 2. Oscillogram of the male call and the female response and the time window during which the female must respond if the male is to approach her to mate.

Figure 3. Mating in *L. punctatissima* 1 – the male has approached close to the female but is not oriented straight towards her.

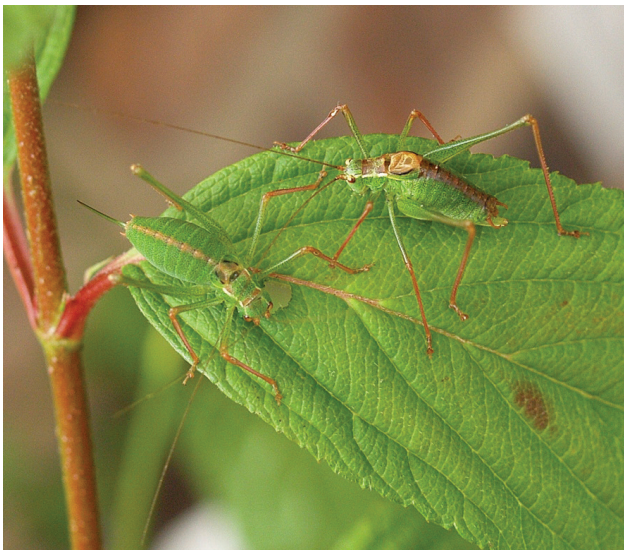

Figure 4. Mating in *L. punctatissima* 2 – the male antennates the female.

Figure 5. Mating in *L. punctatissima* 3 – the male moves in front of the female and backs towards her.

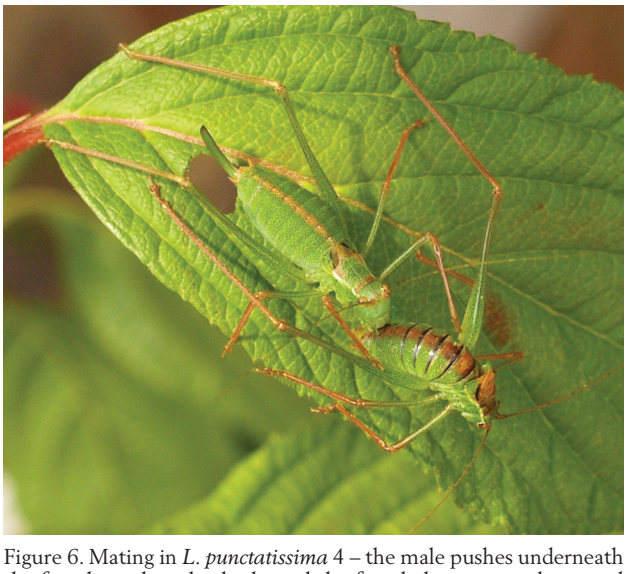

Figure 6. Mating in *L. punctatissima* 4 – the male pushes underneath the female, arching his body, and the female begins to palpate and mouth his back.

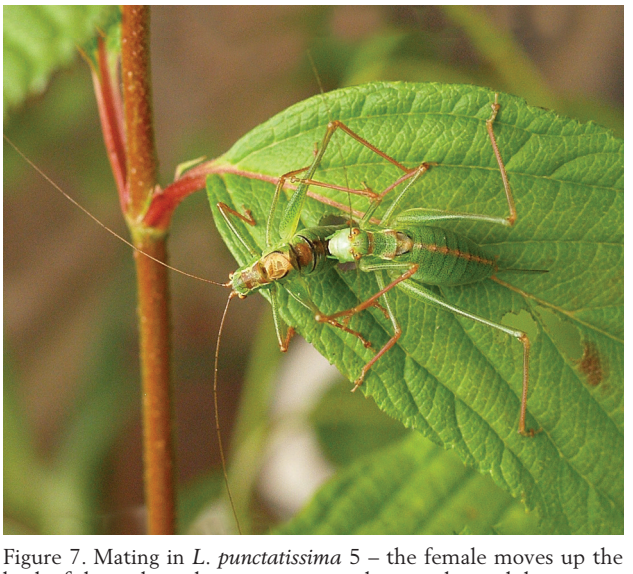

Figure 7. Mating in *L. punctatissima* 5 – the female moves up the back of the male as she continues to palpate and mouth him.

When the male has approached within a few centimetres of the female, often his approach becomes less direct and he appears to be moving more hesitantly, sweeping his antennae around as if trying to find her (Figure 3). We believe that at close proximity phonotaxis may be hampered, with the male possibly having to rely more on cues such as smell or vibration.

Behaviours associated with copulation

Copulation can take place at any time of day in the field and, since we have observed males performing phonotaxis at night, we assume it also takes place at night. When the male finds the female, they explore each other with their antennae (Figure 4). Similar antennation has been widely observed in Orthopteran mating and Ryan & Sakaluk (2009) showed that it is important in sex recognition in decorated crickets, *Gryllodes sigillatus*. Next the male moves in front of the female and backs towards her (Figure 5). He pushes his abdomen underneath her, arching his body, and the female begins to palpate and mouth his back (Figure 6). She gradually moves forward, up the back of the male, as she continues to palpate and mouth him (Figure 7).

If nothing else during this stage, the female must be receiving chemical stimuli from the male. But it is also possible that she is ingesting secretions produced by the male from glands situated on his dorsal tergites that specifically encourage the female to copulate (Gwynne, 2001; Vahed, 1998).

When the female has moved far enough forward so that she is fully mounted over the male, she stops palpating and mouthing his back and bends her abdomen downwards. The male probes the female's genitalia with the tip of his abdomen until he and the female lock genitalia (Figure 8). This is achieved by means of the male's cerci, each of which has an apical tooth that hooks over the lamella and engages with a pit situated on the lateral surface of the base of the female's ovipositor (Vahed *et al.*, 2014). The copulation position shown in Figure 8 has been the same in every mating we have observed. Duncan (1960) described mating taking place with the male and female facing in different directions, but it is unclear how many copulations he actually observed.

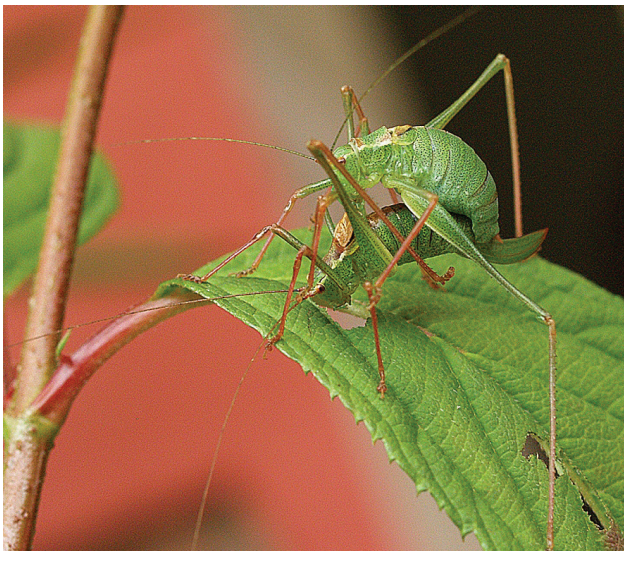

Figure 8. Mating in *L. punctatissima* 6 – the male and female lock genitalia.

At any time up to the point where they lock genitalia, either the male or female may reject their potential mate, either by simply moving away from them or by kicking with the hind legs to push them off. Females are significantly more likely to reject their partner than males are (binomial test, $p<0.0001$). In 249 attempted matings, observed in the lab, where we recorded whether or not rejections took place, successful copulation took place in 173 (69.5%), the female rejected the male in 57 (22.9%) and the male rejected the female in 19 (7.6%). This difference is not because the refractory period (length of time after mating until the individual is willing to mate again) is longer for females than it is for males; we have observed females mating again immediately after they have finished mating, whereas males will not mate again for at least 24 hours.

Like all tettigoniids, *L. punctatissima* produces a nuptial gift in the form of a large, edible spermatophore in which a sperm sac, or ampulla, is surrounded by a spermatophylax consisting of a mass of edible, sperm-free material. In some species, such as *Uromenus stalii*, the spermatophore can be up to 40% of the male's body weight (Gwynne, 2001), but *L. punctatissima* provides a more modest gift averaging only 6.6% in measurements in our lab (SD±2.6%, N=110). The main function of the spermatophyllax is to act as a sperm-protection device, allowing sperm to transfer from the ampulla to the female's spermatheca before she eats the ampulla. In many species of Orthoptera, the spermatophore has very little value as food, but in others it has a parental investment function, providing important nutrients that contribute towards the female's egg production or the survival of her offspring (Gwynne, 2001). In *L. punctatissima* there is, so far, no evidence that the spermatophore has any paternal investment function (Vahed, 2003).

Spermatophore size is largest in the male's first mating (mean 0.013mg, SD±0.004mg, N=37) and gradually decreases in subsequent matings. There is a significant difference between the weight of the spermatophore produced by a male at his first mating compared with his third mating (mean 0.009mg, SD±0.006mg; paired samples t-test, t=-2.323, N=16, $p<0.05$), with no correlation between male size and spermatophore weight. In some other bush-crickets, spermatophore size is related to body condition (e.g. Lehmann & Lehmann, 2009). We measured condition using a slope-adjusted ratio index between overall body length and weight, where this index is independent of size (Jakob *et al.*, 1996). Across the first three matings, combined for all males, the weight of spermatophore produced is significantly correlated with condition just before mating (Spearman rank correlation=0.224, N=100, $p<0.05$), but not with condition just after mating. Nor is there any correlation between condition and the relative size of the spermatophore, i.e. weight of spermatophore as a percentage of body weight. This suggests that spermatophore size is independent of male size and condition, with the correlation with condition pre-mating being due mainly to the effect of the weight of the spermatophore itself on the condition index. Spermatophore weight could depend more on factors such as the length of time since the male's last mating.

The male transfers the spermatophore 2-3 minutes after locking genitalia (Figure 9) and very soon after transfer is complete, the female dismounts from the male by moving forward over him (Figure 10). We recorded the duration of copulation (time from locking genitalia to female dismount) in 169 matings. Copulation lasted from 1min 48s to 5min

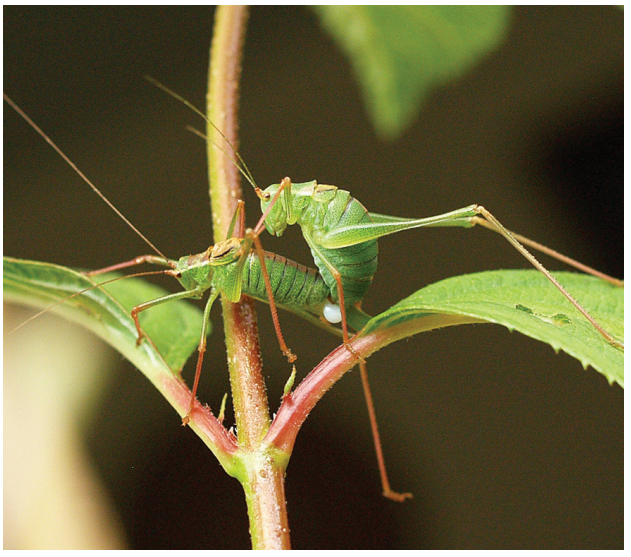

Figure 9. Mating in *L. punctatissima* 7 – the male transfers a spermatophore to the female.

Figure 11. Mating in *L. punctatissima* 9 – after the female dismounts, the male and female gradually move away from each other.

22s (mean 3min 27s, SD±45s). This is very similar to the durations reported for several other bush-cricket species (Samietz *et al.*, 2014; Vahed *et al.*, 2011).

Once the pair have separated they usually wander away from each other (Figure 11), though the female tends to move farther than the male. We have never observed any behaviour that could be interpreted as mate guarding by the male, i.e. behaviour intended to prevent other males trying to mate with the female.

Between 1 and 11 minutes after the pair separate (mean 4.7min, SD±2.0min, *N*=57), and usually close to where the mating took place, the male normally tremulates. In this behaviour, the male performs a series of rhythmic 'push-ups', raising his body and then flexing his legs repeatedly so his body moves up and down quickly several times without actually touching the substrate. He then pauses for at least a few seconds before performing another bout of push-ups. This post-mating tremulation can last for several minutes. It is often performed in the absence of the female and has no effect that we can observe on any other individuals which happen to be nearby at the time. Its function is therefore unclear. Nevertheless, in 68 matings by 17 males where we recorded whether the male tremulated or not, tremulation took place in 89.7% of cases, with all of the males tremulating

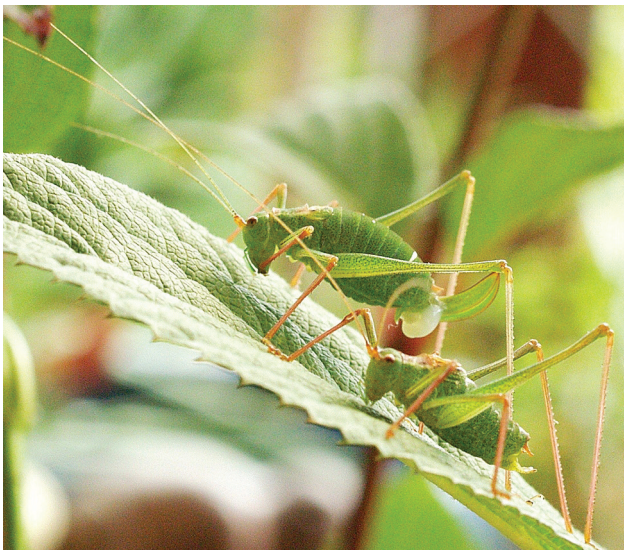

Figure 10. Mating in *L. punctatissima* 8 – when spermatophore transfer is complete, the female dismounts.

for at least some of their matings. Tremulation was only observed in males after copulation and was never observed in females. The only other bush-cricket species we know of where the male usually tremulates after mating is *Leptophyes laticauda* (Vahed, 1994). In other bush-crickets that tremulate, the behaviour seems to have a pre-copulatory function, either to attract females for mating or as part of courtship (Gwynne, 2001). De Luca & Morris (1998), for example, showed that pre-copulation tremulation in meadow katydids provides a reliable indicator of male size and that females prefer larger males. Apart from the two *Leptophyes* species, the only evidence we have found of post-copulatory tremulation in bush-crickets, is for *Copiphora vigorosa*, though it was only observed once (Sarria-S *et al.*, 2016). This species is unusual, however, in that vibration rather than calling is its preferred communication channel, with both males and females tremulating. Male post-copulatory tremulation has, however, been observed as the norm in other Orthoptera (e.g. Brown, 2016; Stritih & Cokl, 2012).

Usually about 20 minutes after the female dismounts (mean 19min 5s, SD±7min 46s, range 6min 16s-45min 10s, *N*=158), she starts to eat the spermatophore. She bends her whole body ventrally so that she can reach the spermatophore with her mouth parts, biting it and pulling strings of it away (Figure 12). Then she slowly consumes the lump she has pulled away before taking another bite (Figure 13). It can take her up to two hours to finish eating the spermatophore (mean 38min 34s, SD±14min 40s, range 12min to 1h 57min, *N*=145).

In 31 cases, we observed males long enough after they mated to record when they started to call again. Even though they do not mate again until at least 24 hours after mating, males may start to call again very quickly. Eighteen males started calling again within an hour of mating and one started only nine minutes after. Males calling soon after mating reject any attempts by females to mate with them. It is unclear why males call when they cannot mate, unless it has some function in male–male competition. However, though we have not studied this systematically, we have not observed nearby males being affected in any obvious way by the calls of a male; they do not appear to move away from him, for example.

Although we have observed only 10 or so copulations in the field, we have never noticed any differences between the

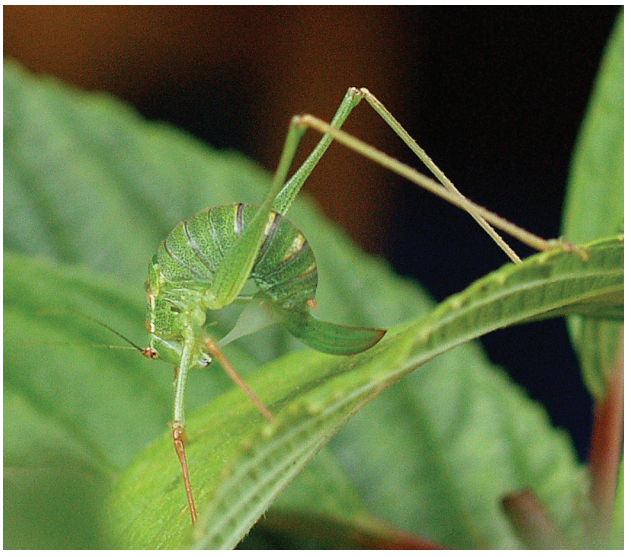

Figure 12. Mating in *L. punctatissima* 10 – the female bends to bite the spermatophore and pull away pieces.

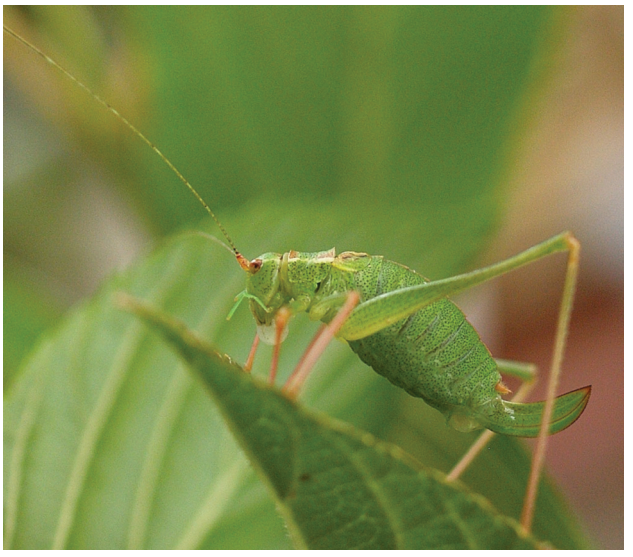

Figure 13. Mating in *L. punctatissima* 11 – the female eats a piece of the spermatophore.

behaviours observed in the lab and those seen in the wild. Most of the behavioural elements associated with copulation described here for *L. punctatissima* have also been observed in other tettigoniids (e.g. Dorkova *et al.*, 2019) or other Orthoptera (e.g. Field & Jarman, 2001). The only unusual behaviour in *L. punctatissima* is possibly tremulation occurring as the norm after copulation. This may be limited to the genus *Leptophyes*. We would therefore be interested to hear if anyone has observed it in another bush-cricket genus, or another species of *Leptophyes*.

Acknowledgements

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